2020

The Use of Oncolytic Viruses in Tumor Therapy

Duncan Webb

The Use of Oncolytic Viruses in Tumor Therapy

Duncan Webb
Department of Mathematics
Xavier University
Cincinnati, OH 45207-4441

Hem Raj Joshi
Department of Mathematics
Xavier University
Cincinnati, OH 45207-4441

Abstract

Cancer is a term used for a classification of diseases in which abnormal cells divide without control and invade other tissues. Cancer cases have been at an all time high as of 2020, with an estimated 1.7 million new cases in 2019 alone. Due to the severity and lethality of this type of disease, many billions of dollars have been invested in finding a cure, but one is yet to be obtained. A newer form of tumor therapy to help with this disease is being researched, called oncolytic virus therapy. An oncolytic virus is one which specifically targets cancerous cells using proteins expressed on the outside of only infected cells. In this paper, we expand on a simple oncolytic virus therapy model created by Wodarz to model an agent-based system where no spatial restrictions are in place, infections are driven by laws of mass-action, and we assume a simple in-vitro environment. We evaluate these models in an ideal environment with qualitative and quantitative analysis, and compare the result to the results found by Wodarz in the in-vitro simulations he performed. We conclude with an evaluation of when the model is effective for the simulation of a spatially sensitive system, and attempt to explain the mathematical complexities with those cases where it's ineffective.

1 Biological Background

Cancer, or malignancy, is an abstract term collecting all diseases which display an abnormal growth of cells. In every type of cancer, some of the body's cells begin to divide without stopping and spread into the surrounding tissues. In the body, cells typically grow and divide to form new cells when the body needs them to replace damaged or dead cells. Cancerous cells may survive when they should die, and begin to form new cells when they're not needed. These cells may divide further without need and form growths on the body called tumors.

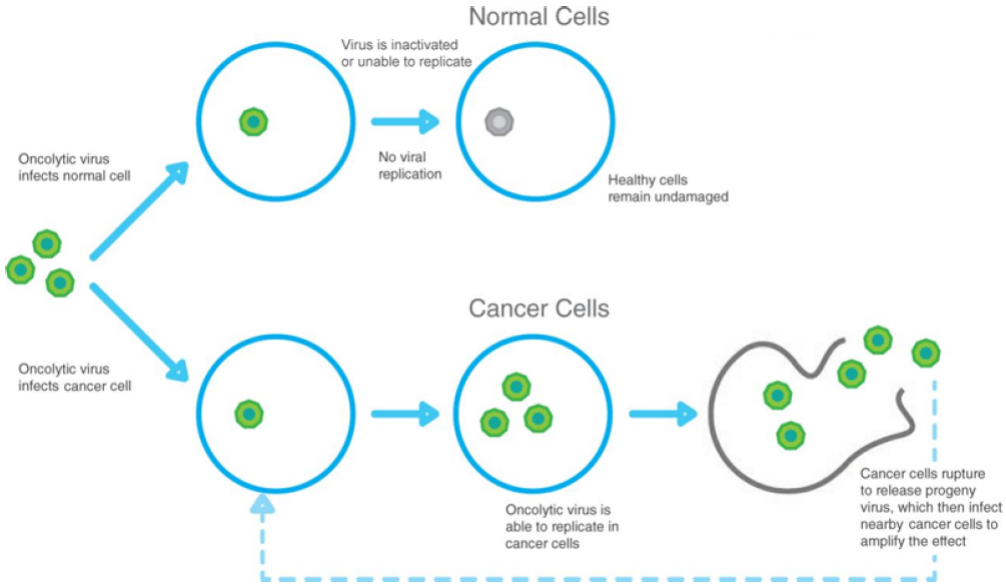
Most cancer types are genetic diseases caused by mutations within certain genes which often allow cells to ignore signals which regulate cell growth and apoptosis. The three main types of genes which contribute to cancer growth when modified would be the proto-oncogenes, tumor suppressor genes, and DNA repair genes. Proto-oncogenes are the normal cell growth and division genes which become more active when a cancer causing mutation exists. Tumor suppressor genes are genes which contribute to the regulation of cell growth when excess division occurs. DNA repair genes are genes responsible for fixing damaged DNA, and with the restriction of this gene's expression comes the ability for more mutations to occur[5].

When any combination of these three types of genes are mutated, cancer often ensues. Along with the cancerous mutations, cells develop genetic impairments in their antiviral defenses which make them susceptible to infection.

In this new form of immunotherapy, we take advatage of this susceptibility and engineer viruses to target cancer cells. These engineered viruses, called oncolytic viruses, are modified versions of viruses such as hepatitis B virus(HBV) or human papilloma virus(HPV) which are intentionally designed to attack tumors which have already formed. Within these viruses immune-stimulating genes are inserted and targets of the tumerous cells are removed.[1] The oncolytic effect can take three distinct modes of virus-host interactions. One form would be repeated cycles of virus replication in the tumor, where the virus replicates itself within the

tumor and leads to cell death and tumor reduction. Another would be a low reproductive rate with a highly cytotoxic protein being produced to damage the cell. The last form would be virus infection of cancer cells which induce antitumor immunity. This infection causes inflammation and lymphocyte penetration into the tumor, with the virus antigens eliciting increased sensitivity to tumor necrosis factor-mediated killing.[6]

Figure 1: The Oncolytic Viruses invading normal cells and cancer cells[3]

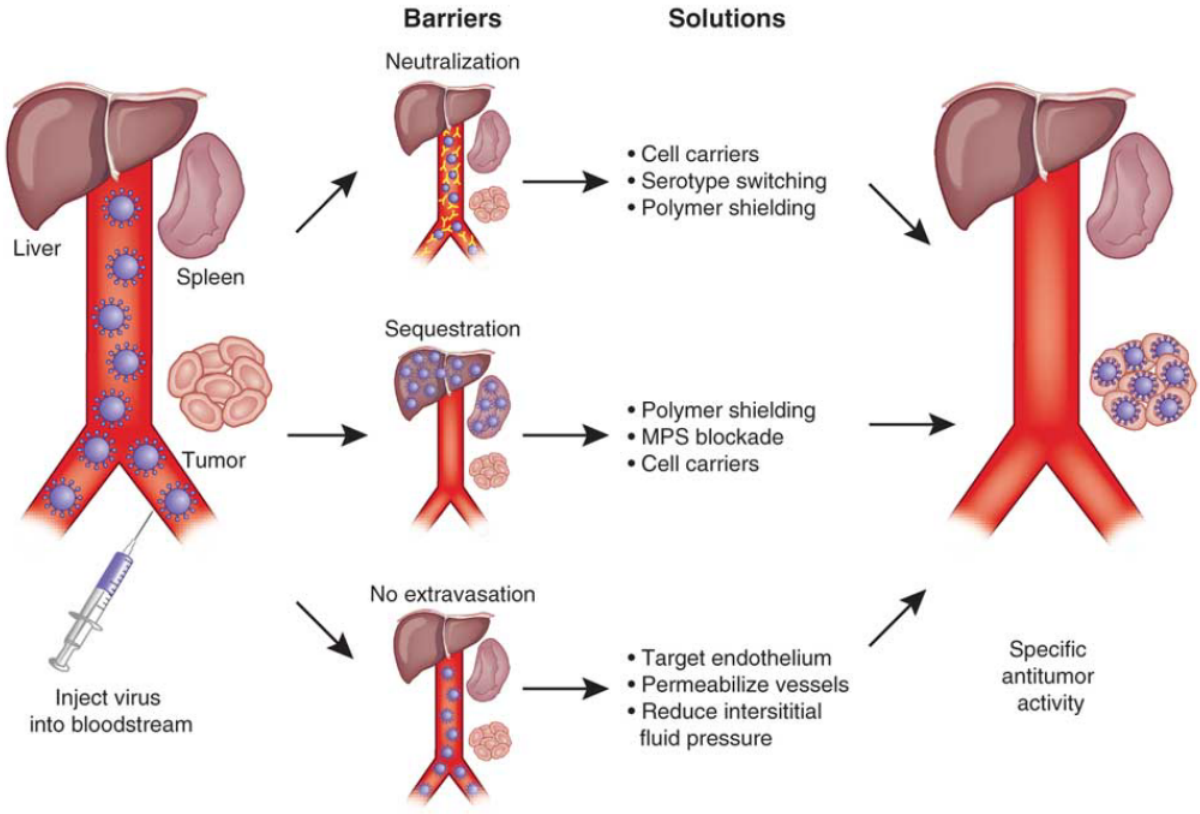


Within this paper we will be discussing the differences between a virus which is highly cytotoxic but slowly replicating, and one whcih is less cytotoxic and replicating faster. The ultimate goal is to eradicate the non-infected cancerous cells within a tumor. When considering the best option between these two, we must also consider the effects of cancer on the immune system as well as the dangers of introducing a new disease into the body in doses large enough to kill a tumor or to replicate to become that large. Cancers often inhibit the immune response in the body, weakening the immune system and making the host more susceptible to new diseases. Therefore, introducing a new virus which the body is unfamiliar with can cause dangerous side effects. This is why the viral cells should only be able to replicate within the environment of the tumor cells, and spread only through the tumor.

This problem leads to another question, where should the virus be injected? When in-

jected into the bloodstream, the virus will run into various barrier provided by the weakened, but still functioning, immune system.

Figure 2: Visualization of the liver interacting with an oncolytic virus when injected into the bloodstream[3]



It can be seen that the liver and the spleen can sequester a large amount of the virus dosage and remove it from the body through natural excretion methods, significantly reducing the amount of the dosage which is applied to the cancerous cells they are meant for. Some viruses have been clinically proven to evade antibodies, while others may remain infectious to the target cells while being bound to antibodies. Others may be engineered to infect cells of the immune system and be transferred by the bloodstream to the cancerous cells. The most promising mode of delivery, however, is the use of cell carriers. This is the case because they provide protection from the immune system, passing through it undetected.[3]

2 The Model

Many mathematical models have been created around this subject, specifically attempting to analyze the efficiency by which the oncolytic viruses target the cancerous cells. In a paper by Wu, Kirn, and Wein[9], the model presented takes into account the immune response to the administered virus by introducing the tumor as a mass of constantly distributed liquid particles wherein the cells move by convection with a velocity function dependent on the radial distance from the center and the time from the beginning of simulation.

We begin our modeling with a simple ordinary differential equations model described by Wodarz [8] which is derived from the agent based model which expresses all infection and reproduction events driven by the Law of Mass Action:

$$\begin{aligned} \frac{dS(t)}{dt} &= rS(t)\left(1 - \frac{S(t)+I(t)}{K}\right) - \frac{\beta S(t)I(t)}{K} \\ \frac{dI(t)}{dt} &= \frac{\beta S(t)I(t)}{K} - aI(t) \end{aligned} \tag{1}$$

Where the number of uninfected tumerous cells at time t is denoted by S(t), and the number of infected cells at time t by I(t). K here is a carrying capacity, usually determined by the size of the tumor and the number of cancerous cells within it. r represents the reproductive rate of cancerous cells, limited to the carrying capacity of the system by $\left(1 - \frac{S(t)+I(t)}{K}\right)$. β is the infection rate due to interaction between S(t) and I(t). a is the death rate of the infected cells, where the average lifespan of an infected cell is 1/a.This modified Lotka-Volterra system[7] is a very well understood mathematical model with plenty of documentation regarding the qualitative analysis, which we will return to later.

The model presented by Wozard can be used to describe a spatially sensitive environment by developing a simulation in which uninfected cells and infected cells are allowed to expand in space. Wozard ran 7 simulations, all of which began with the environment being a 300x300 grid of cells wherein a 5x5 block of cancerous infected cells are placed in the middle of a 13x13 block of uninfected cells. From this we gain our initial condition for the analysis of

the model. That is:

$$\begin{aligned} S(0) &= 169 \\ U(0) &= 25 \\ K &= 90000 \end{aligned} \tag{2}$$

Even though the spatial stochastic predator-prey system studied here shows many patterns, the dynamics shown in all of them can be understood by studying the local interactions of the agents. According to Pascual et. al., in a class of systems exhibiting oscillatory dynamics, the functional forms governing the local predator-prey interactions at those characteristic scales are the same as the ones describing a perfectly mixed, mass-action system. [8]

3 Analysis

In analysis of this system, we first use the method defined in the Castillo-Chavez Paper[2] to determine the equilibria of which there are three:

$$\begin{aligned} (S^0, I^0) &= (0, 0) \\ (S^1, I^1) &= (K, 0) \\ (S^2, I^2) &= \left(\frac{aK}{\beta}, \frac{rK\left(\beta - a\right)}{\beta\left(r + \beta\right)} \right) \end{aligned} \tag{3}$$

In the first equilibria, the populations are both extinct. We call this the trivial equilibrium since it biologically implies that no susceptible cells exist. In the second equilibria, the uninfected population persists at the carrying capacity and the virus population is extinct. We call this the disease free equilibrium. The third equilibria demonstrates where the virus successfully infects the tumor and spreads until an equilibrium is reached. We call this an endemic equilibrium since the susceptible and infected classes both exist. This third equilibrium is stable if $a < \beta$, and can be approached monotonically or involve a system of dampened oscillations.

Next, we use the method of linearization to reduce the system of non-linear ordinary differential equations to a system of linear differential equations, in order to estimate the solution of the equation for a given time value t. To do this, we first determine the fixed points of our system. We know from the above analysis that the system has three fixed points defined to be the equilibria. We will use this linearization to perform stability analysis as described in [4]. Now let:

$$f_1(\vec{x}(t)) = rS(t)\left(1 - \frac{S(t)+I(t)}{K}\right) - \frac{\beta S(t)I(t)}{K}$$

$$f_2(\vec{x}(t)) = \frac{\beta S(t)I(t)}{K} - aI(t) \tag{4}$$

where

$$\vec{x}(t) = \begin{pmatrix} S(t) \\ I(t) \end{pmatrix}$$

represents the functions we look to approximate the numbers for. Let $\vec{a_1}, \vec{a_2}$, and $\vec{a_3}$ be the three fixed points vectors. Then we set up the system:

$$\vec{y(t)} = \vec{x(t)} - \vec{a} \tag{5}$$

Which by differentiation implies:

$$\frac{d\vec{y(t)}}{dt} = \frac{d\vec{x(t)}}{dt} = \begin{pmatrix} f_1(\vec{x}(t)) \\ f_2(\vec{x}(t)) \end{pmatrix} = f(\vec{x(t)}) = f(\vec{x(t)}) + f(\vec{a})$$

Since $f(\vec{a})$=0 by definition. Then we can approximate towards linearization:

$$\frac{d\vec{y(t)}}{dt} = f(\vec{x(t)}) + f(\vec{a}) \approx \left(df(\vec{a})\right)(\vec{x} - \vec{a}) = J\vec{y}$$

where J represents the Jacobian matrix of the above system evaluated at each of the fixed points. For our model, the Jacobian matrix is

$$J(\vec{x(t)}) = \begin{pmatrix} \frac{rK - 2rS(t) - (r+\beta)I(t)}{K} & \frac{(r-\beta)S(t)}{K} \\ \frac{\beta I(t)}{K} & \frac{\beta S(t) - aK}{K} \end{pmatrix}\Bigg|_{\vec{x(t)}=\vec{a}} \tag{6}$$

The solution for this system is found to be

$$y(t) \approx \sum_{i=1}^{6} C_i e^{\lambda_i t} \vec{\lambda_i} \tag{7}$$

where λ_i and $\vec{\lambda_i}$ are the ith eigenvalue and eigenvector of the matrix J respectively. We will look at each solution in the summation to predict the behavior of each. In our model, there are three cases for $\vec{a}$.

Case 1 $\vec{a} = \vec{a_1}$. Then the J matrix becomes:

$$J = \begin{pmatrix} r & 0 \\ 0 & -a \end{pmatrix}$$

who has eigenvalues:

$$\lambda_1 = r$$

$$\lambda_2 = -a$$

and respective eigenvectors:

$$\vec{\lambda_1} = C_1 \begin{pmatrix} 1 \\ 0 \end{pmatrix}$$

$$\vec{\lambda_2} = C_2 \begin{pmatrix} 0 \\ 1 \end{pmatrix}$$

Since we assumed non-zero positive real values for the coefficients of the equations, we know λ_1 is positive and λ_2 is negative. Regardless of the values of r and β, this fixed point will be a saddle point.

Case 2 $\vec{a} = \vec{a_2}$. Then the J matrix becomes:

$$J = \begin{pmatrix} -r & -(r+\beta) \\ 0 & r-a \end{pmatrix}$$

who has eigenvalues:

$$\lambda_3 = -r$$

$$\lambda_4 = r - a$$

and respective eigenvectors:

$$\vec{\lambda_3} = C_3 \begin{pmatrix} 1 \\ 0 \end{pmatrix}$$

$$\vec{\lambda_4} = C_4 \begin{pmatrix} \frac{r+\beta}{a+2r} \\ 1 \end{pmatrix}$$

By the same logic as above, we know λ_3 is negative. Here, we have 3 cases for what the fixed point will be classified as:

Subcase 1: $r < a$. In this case, the second eigenvalue will also be negative. This would result in the fixed point being a stable node.

Subcase 2: $r > a$. In this case the second eigenvalue will be positive. This would result in a saddle point as well.

Subcase 3: $r = a$. In this case the second eigenvalue will be zero. There will be no motion in regards to the second eigenvalue. Instead, it will run parallel to the first eigenvalue.

Case 3 $\vec{a} = \vec{a_3}$. Then the J matrix becomes:

$$J = \begin{pmatrix} \frac{r\left(K - \frac{2Ka}{\beta} - \frac{K\beta r - Kar}{\beta^2 + \beta r}\right) - \frac{\beta(K\beta r - Kar)}{\beta^2 + \beta r}}{K} & -\frac{(r+\beta)a}{\beta} \\ \frac{\beta(K\beta r - Kar)}{K(\beta^2 + \beta r)} & \frac{\frac{rKa}{\beta} - aK}{K} \end{pmatrix}$$

who has eigenvalues:

$$\lambda_5 = \frac{-\frac{1}{2}a\beta + \frac{1}{2}\sqrt{a^2\beta^2 + 4a^2r^2 - 4a\beta^2 r}}{\beta}$$

$$\lambda_6 = \frac{-\frac{1}{2}a\beta - \frac{1}{2}\sqrt{a^2\beta^2 + 4a^2r^2 - 4a\beta^2 r}}{\beta}$$

and respective eigenvectors:

$$\vec{\lambda_5} = C_5 \begin{pmatrix} \frac{(r+\beta)a}{-\frac{1}{2}a\beta+\frac{1}{2}\sqrt{a^2\beta^2+4a^2r^2-4a\beta^2r}+ra} \\ 1 \end{pmatrix}$$

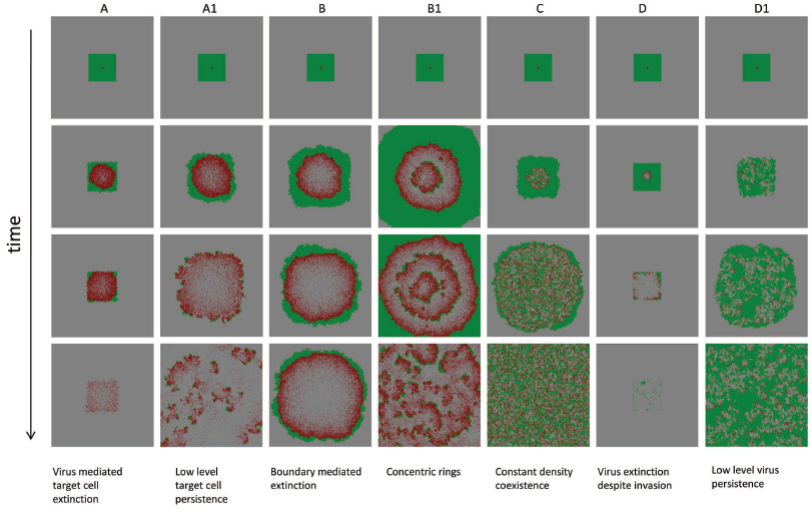
$$\vec{\lambda_6} = C_6 \begin{pmatrix} 1 \\ \frac{(r+\beta)a}{-\frac{1}{2}a\beta-\frac{1}{2}\sqrt{a^2\beta^2+4a^2r^2-4a\beta^2r}+ra} \end{pmatrix}$$

This pair of eigenvalues has a variety of classifications based on the values of a, β, and r.

Subcase 1: $a^2\beta^2 + 4a^2r^2 \geq 4a\beta^2r$ This case implies the eigenvalues are both real numbers. In this case, we know λ_6 is always negative. If $\sqrt{a^2\beta^2 + 4a^2r^2 - 4a\beta^2r} > a\beta$, then λ_5 is positive and the fixed point is a saddle point. If $\sqrt{a^2\beta^2 + 4a^2r^2 - 4a\beta^2r} < a\beta$, then λ_5 is negative and the fixed point is a stable node. If $\sqrt{a^2\beta^2 + 4a^2r^2 - 4a\beta^2r} = a\beta$ then λ_5 is 0 and we have the same parallel motion as described above.

Subcase 2: $a^2\beta^2 + 4a^2r^2 < 4a\beta^2r$. This case implies both eigenvalues are complex numbers. In this case, we would have negative real parts in both so we have exponential decay in a stable spiral.

Figure 3: The result of Wozard's simulation [8]



This figure shows 4 snapshots in time in the agent based model with the parameters r, β, and a being modified. The selected values for each simulation are given:

Simulation	r	β	a
Simulation A	0.013	0.14	0.003
Simulation A1	0.15	0.32	0.007
Simulation B	0.014	0.015	0.00056
Simulation B1	0.04	0.032	0.016
Simulation C	0.014	0.032	0.008
Simulation D	0.0002	0.019	0.0032
Simulation D1	0.069	0.64	0.18

We can see a variety of interaction types form. In A, we see an execution of a virus-mediated target cell-extinction system. In this case, both the targeted cell and the virus is spreading in space as a wave, but the virus infection ring overtakes the uninfected replication ring, causing the uninfected cells to disappear. The virus is strong enough to eliminate the wave. In A1, we see a modified version of A where the disease spreads similarly, but only partially breaks up the cell wave. The structure allows uninfected cells to spread out in all directions and cause new moving fronts. We call this Low level target cell persistence.

The coefficient values in A cause the disease free equilibrium to be a stable node and the endemic equilibrium to be approached in a stable spiral. Due to the initial conditions, our solution approached the endemic equilibrium. The coefficient values in A1 cause the disease free equilibrium to be a saddle point, and the endemic equilibrium to be approached in a stable spiral. We see similar behaviors expressed in the Wodarz simulation in Figure 4.

In B, we see a boundary mediated extinction scenario, where the infection and the growth occur at almost the exact same rate. The rings spread but the virus never overtakes the growth. This leads to cell extinction when the boundary is reached. Since it is possible for real tumors to break out of the carrying capacity of their environment, the genetic transformations needed to do so take place on a longer time scale, so it is reasonable to assume some sort of geometric constraints. B1 expresses a concentric ring pattern tending to target cell persistence at low levels. This is due to the existence of uninfected cells left

behind by the expanding ring of virus cells. The existence here leads to new target cell growth, followed by new virus growth, leading to new wave structures to form.

The coefficient values in B cause the disease free equilibrium to be a saddle point and the endemic equilibrium to be approached in a stable spiral. Due to our initial conditions, the simulation approaches the endemic equilibrium, unlike the natural simulation. The coefficient values of B1 cause the disease free equilibrium to be a saddle point and the endemic equilibrium to be approached in a stable spiral. Our initial conditions cause the solution to approach the endemic equilibrium.

In C, we see a constant density coexistence between the virus and the uninfected cells. As the virus spreads in space, many uninfected cells are left uninfected, leading to the dispersion of the initial expansion and the lack of waves. This is the well mixed system which reaches an equilibrium density after a finite amount of time.

The coefficient values in C cause the disease free equilibrium to be a saddle point and the endemic equilibrium to be approached in the stable spiral. Our initial conditions make the simulation approach the endemic equilibrium, which makes sense biologically.

In D, we see a virus extinction despite having invaded the population. The virus reduces the cancer load by a small amount, and dies off in the process. The cancer is then left to grow unopposed. This result is less likely to occur when the virus strength increases, which is quantified by the ratio a/β. In D1, we see low-level virus persistence. While the virus invades the target cell population, it also converges to its local equilibrium value.

The coefficient values of D cause the disease free equilibrium and the endemic equilibrium to be stable nodes. Our initial conditions tend to the disease free equilibrium. The coefficients in D1 cause the disease free equilibrium to be a stable node and the endemic equilibrium to be approached in a stable spiral. Our initial conditions cause the simulation to tend towards the endemic equilibrium.

Overall, we can see the model is effective in recreating the actual results for the 300x300 in-vitro experiment run by Wozard.

4 Conclusions

The use of Oncolytic Viruses in Tumor Therapy can be a very powerful tool in the study of immunotherapy. This review employed standard qualitative analysis techniques to generalize the behavior, as well as standard quantitative analysis to demonstrate where the model can successfully be used to demonstrate the natural long term behavior of the disease.

The use of ordinary differential equations is employed often in the field of virus dynamics and yield many useful results. With these models comes weaknesses in the predictive power in situations such as these. In reality, it's almost impossible to exactly predict the natural world due to the random behavior exhibited in nature. These models also are in-effective in exactly describing an in-vivo environment for the same reason. Even with these weaknesses, ordinary differential equation models can be powerful in describing ideal behaviors of these environments.

Other research done with these models include validating these models with scientific experiments as done by Wodarz, and introducing additional biological complexities with the goal of creating a biologically realistic model which can be used to develop treatment strategies for diseases which previously had no cure in sight.

5 Figures

Figure 4: The result of maple simulation using simulation A values

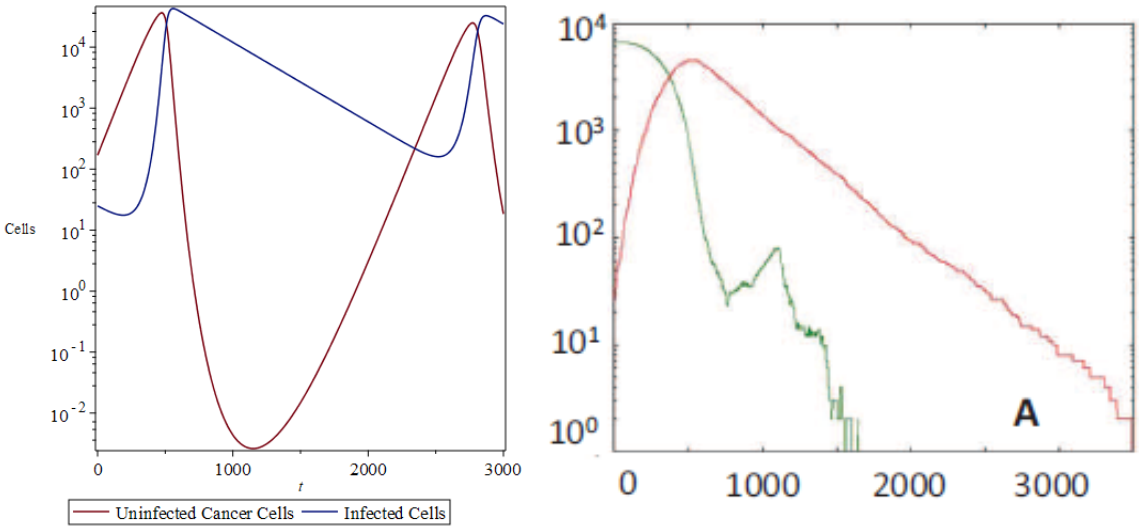


Figure 5: The result of maple simulation using simulation A1 values

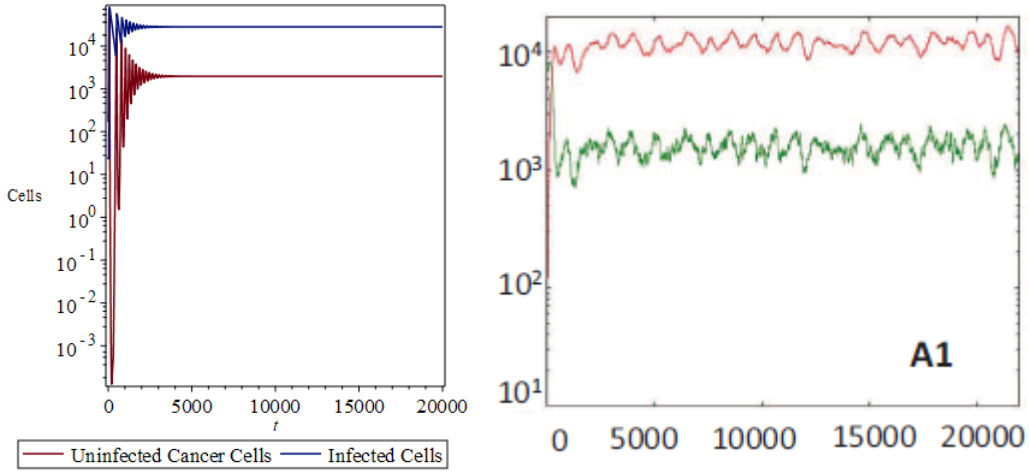


Figure 6: The result of maple simulation using simulation B values compared to an experiment run by Wodarz

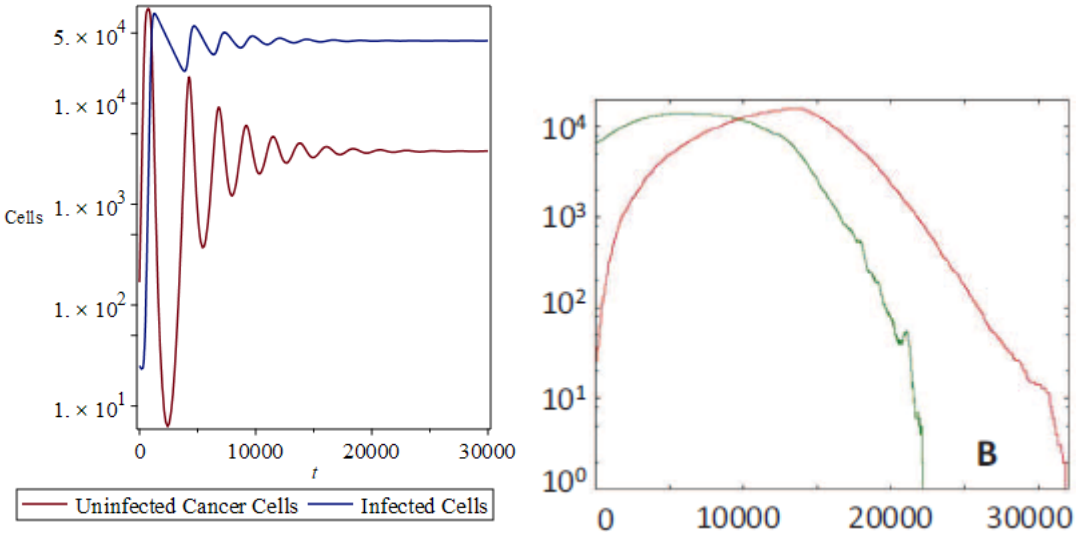


Figure 7: The result of maple simulation using simulation B1 values compared to an experiment run by Wodarz

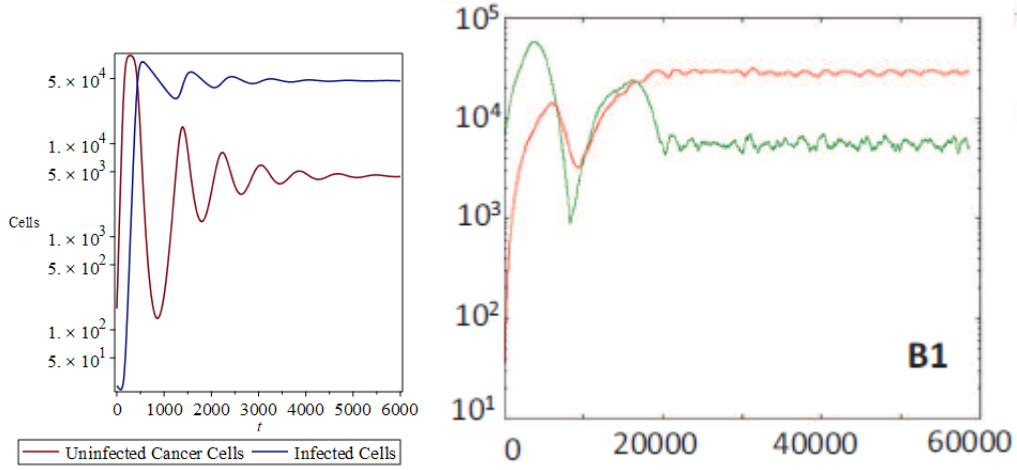


Figure 8: The result of maple simulation using simulation C values compared to an experiment run by Wodarz

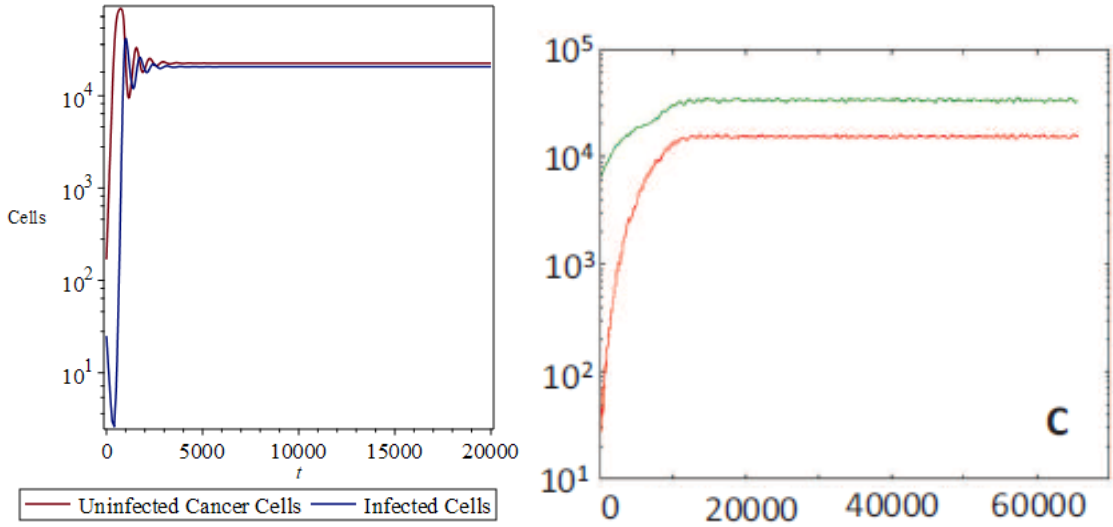


Figure 9: The result of maple simulation using simulation D values compared to an experiment run by Wodarz

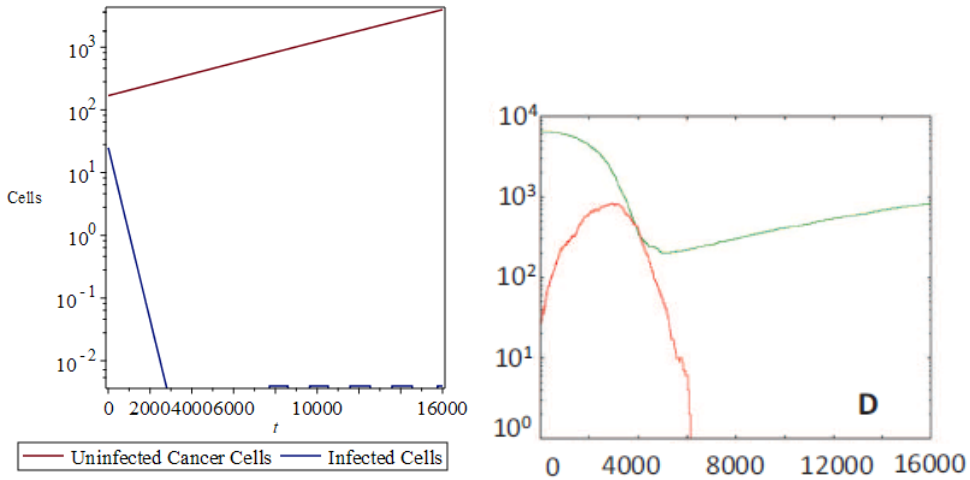
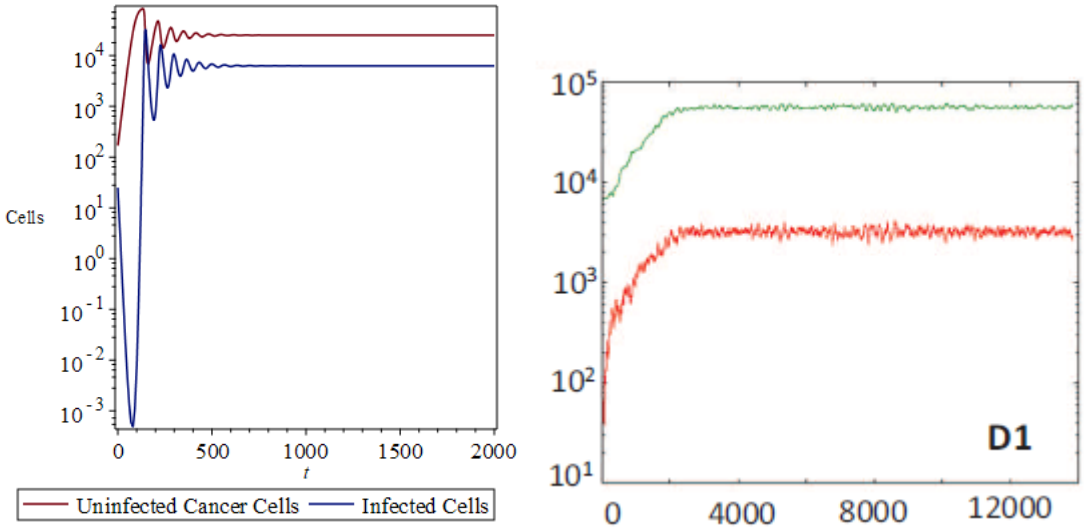


Figure 10: The result of maple simulation using simulation D1 values compared to an in-vitro experiment run by Wodarz



References

[1] Bell, John C. *How Oncolytic Virus Therapy is Changing Cancer Treatment* Cancer Research Institute, 2020

[2] Castillo-Chavez, Feng, and Huang *On the Computation of R_0 and its Role on Global Stability* IMA, 2001

[3] Han, Nate and Joshi, Hem Raj, *Characteristics of Oncolytic Viruses Influencing Effectiveness as a Tumor Therapy*, Xavier University, 2014

[4] Lloyd, Alun. *Linearization of Differential Equation Models*, 2017

[5] National Cancer Institute, *What is Cancer?*, https://www.cancer.gov/about-cancer/understanding/what-is-cancer, 2015

[6] Novozhilov, Artem et. al., *Mathematical modeling of tumor therapy with oncolytic viruses: Regimes with complete tumor elimination within the framework of deterministic models* Biology Direct, 2006

[7] M.A. Nowak and R. M. May, "Virus Dynamics. Mathematical Principles of Immunology and Virology", 2000: Oxford University Press.

[8] Wodarz, Dominik, *Computational Modeling Approaches to Studying the Dynamics of Oncolytic Viruses*, Mathematical Biosciences and Engineering, Vol. 10, Num. 3, 2013

[9] Wu, J.T. , Wein, L. M., et al. *Validation and analysis of a mathematical model of a replication-competent oncolytic virus for cancer treatment: Implications for virus design and delivery*, Cancer res., **63** (2003), 1317-1324